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Convergent evolution of carrion and faecal scent mimicry in fly-pollinated angiosperm flowers and a stinkhorn fungus

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Abstract

Flowers of many angiosperms attract fly pollinators through mimicry of animal carrion and faeces. This phenomenon of "sapromyiophily" is also evident in the sporophytes of some mosses and fruiting bodies of "stinkhorn" fungi, both of which use flies as agents of spore dispersal. We studied the scent chemistry of a stinkhorn fungus (*Clathrus archeri*) and seven fly-pollinated plant species with foetid odours to determine the degree to which these organisms mimic the scent of carrion and faeces (reference scent samples were collected from rotting meat, a rat carcass and horse and dog faeces), as well as the degree of convergent evolution between the fungus and angiosperm flowers. We found that scents of both the fungus and angiosperms tended to contain compounds typical of carrion, such as oligosulphides, and of faeces, such as phenol, indole and p-cresol. This study provides compelling new evidence for mimicry of carrion and faeces, as well as a striking pattern of convergence in the putrid scents of the fungus and the angiosperms, relative to those of confamilial species. The syndrome of sapromyiophily thus encompasses at least two kingdoms (Plantae and Fungi) and provides an effective means of exploiting flies as agents of pollen and spore dispersal.

Keywords: Brood-site mimicry; Calliphoridae; *Clathrus archeri*; Diptera; Flower scent; GC-MS; Intraspecific variation; Sapromyiophilous; Stapeliads

1. Introduction

Floral "pollination syndromes" (sensu Faegri and Van der Pijl, 1979) provide classical examples of convergent evolution — similarity of traits in unrelated organisms that have adapted to similar environmental factors. These syndromes, which arise when unrelated plants adapt to the same functional pollinator group (Fenster et al., 2004), are usually described using qualitative characteristics of the morphology, colour and scent of flowers (Ollerton et al., 2009). However, general descriptors of floral scent such as "sweet", "pungent" etc. are inadequate to describe the chemical complexity of this phenotypic trait (Raguso, 2008). An increasing number of studies thus use coupled gas chromatography-mass spectrometry (GC-MS) for quantitative investigations of floral scents (Knudsen et al., 2006), but there are still comparatively few of these that explicitly consider the hypothesis of convergent evolution in scent chemistry among unrelated plants that share pollinators (Jürgens et al., 2006; Knudsen and Tollsten, 1993, 1995).

"Sapromyiophilous" flowers – those which attract carrion and dung flies through mimicry of their food and brood sites – have evolved in many angiosperm families (Banziger and Pape, 2004; Burger et al., 1988; Johnson, 1997; Kite et al., 1998; Ollerton and Raguso, 2006; Stensmyr et al., 2002; Shuttleworth and Johnson, 2010a, 2010b). These species have foul-smelling flowers which are typically brown with purple or reddish blotches and often unusually large, as exemplified by *Stapelia gigantea* (Apocynaceae) (this study) and *Rafflesia arnoldii* (Rafflesiaceae) (Barkman et al., 2008). There is now good evidence that the attraction of flies to these flowers depends heavily on the emission of volatiles that are used by flies as cues to locate carrion, faeces and even urine (Shuttleworth and Johnson, 2010a, 2010b; Stensmyr et al., 2002). Stensmyr et al. (2002) showed that oligosulphides emitted by the Mediterranean 'dead-horse' *Arum* were identical to those emitted by animal carrion and triggered the same electrophysiological response in fly antennae. More recently, Shuttleworth and

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Johnson (2010a, 2010b) demonstrated that a shift to pollination by carrion flies could be induced in wasp-pollinated *Eucomis* species (Hyacinthaceae) simply by experimental addition of oligosulphides to inflorescences. Investigations of the chemical composition of the scent of sapromyiophilous flowers by Kite and Hetterscheid (1997) and Jürgens, et al. (2006) indicate that there may be a number of different strategies in operation, including mimicry of carrion (characterized by oligosulphide emission), mimicry of faeces (characterized by emission of p-cresol, phenol and skatole) and mimicry of urine (various acids). However, the patterns are not yet clear and sampling of more species and manipulative experiments are required to gain increased understanding of the significance of particular volatile blends. South Africa with its rich diversity of carrion flowers, particularly among succulent stapeliads (Bruyns, 2005; Meve and Liede, 1994), provides ideal opportunities for this purpose.

Attraction of flies through mimicry of their food and brood sites is not confined to angiosperms. There is now good evidence that sapromyiophily occurs among both mosses and fungi (Fischer and Vicha, 2003; Marino et al., 2009), although the function of fly attraction in these cases is for subsequent dispersal of spores rather than gametes. In mosses, sticky spores are usually transported on the bodies of flies (Marino et al., 2009), while in fungi spores are often consumed along with an exudate (the "gleba") and germinate once they have passed through the digestive system of flies (Tuno, 1998). Stinkhorn fungi belonging to the Phallaceae have foul-smelling fruiting bodies that are known to attract flies (Fischer and Vicha, 2003; Sleeman et al., 1997; Tuno, 1998). This study

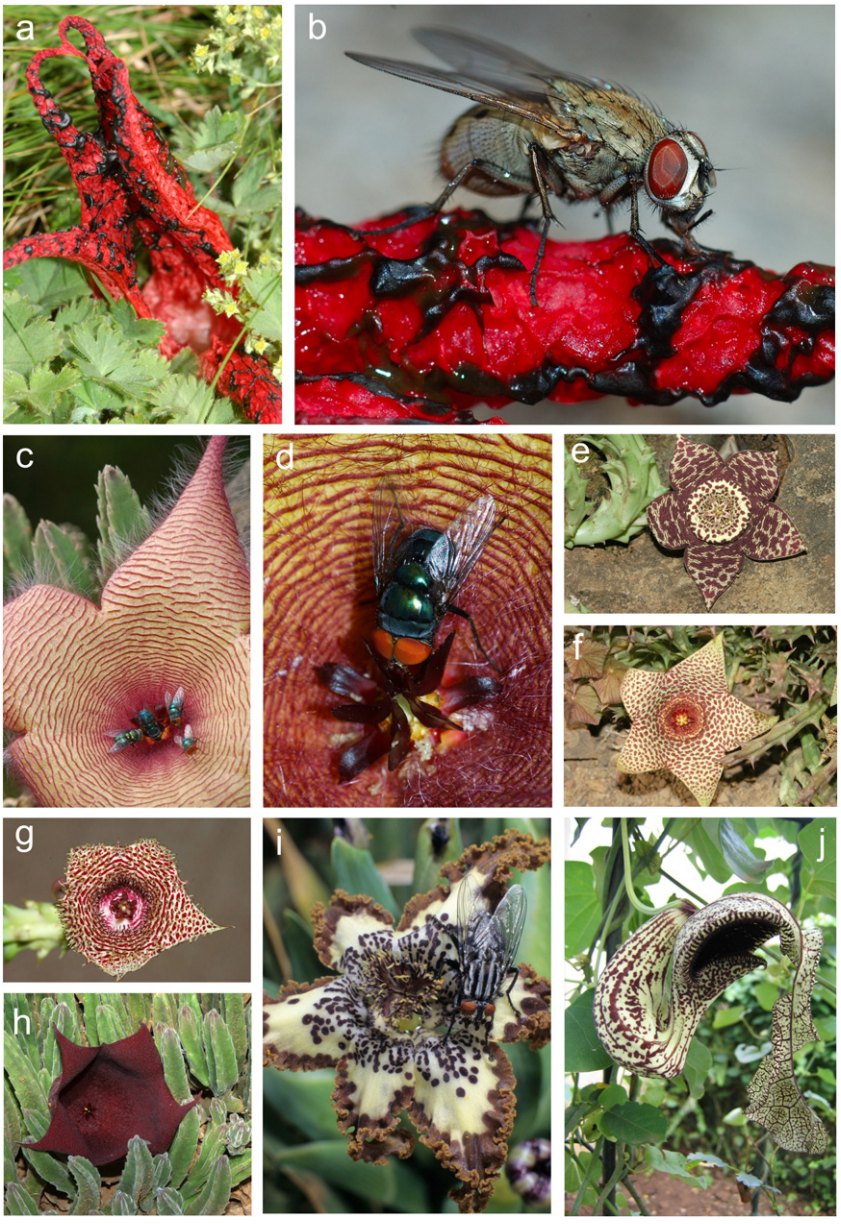

Fig. 1. Sapromyiophilous species included in the scent analysis: (a) Fruiting body of the stinkhorn fungus *Clathrus archeri*; (b) Muscid fly feeding on the gleba of *C. archeri*; (c). *Stapelia gigantea* and visiting calliphorid flies; (d). Gynostegium of *S. gigantea* with eggs freshly deposited by a calliphorid fly; (e) *Orbea variegata*; (f) *Orbea verrucosa;* (g) *Huernia hystrix*; (h) *Stapelia leendertziae*; (i) *Ferraria crispa* with visiting sarcophagid fly; (j) *Aristolochia cymbifera*.

includes an investigation of the scent of *Clathrus archeri*, a species native to Africa and Australasia (Dring, 1980) which is commonly known as the octopus or cuttlefish stinkhorn (Fig. 1a). Our preliminary observations indicate that the foul-smelling fruiting bodies of this species are visited by numerous flies, including blowflies (Calliphoridae), flesh flies (Sarcophagidae) and houseflies (Muscidae) (Fig. 1b). The scent of this species has not been investigated previously, but oligosulphides typical of carrion have been shown to be emitted from fruiting bodies of another stinkhorn, the European species *Phallus impudicus* (Borg-Karlson et al., 1994). Although the gleba of stinkhorns has some nutrient value, investigations of the North American species *Mutinus caninus* show that this is far less than that which flies obtain from dung or faeces (Stoffolano et al., 1990). We are also not aware of any evidence to suggest that the short-lived fruiting bodies of stinkhorn fungi are used as a brood place for the larvae of necrophagous flies. The system can thus be described as one of deception.

In this study we investigated the scent of the stinkhorn *C. archeri* and several angiosperm species that have flowers which are known or suspected to be pollinated by carrion flies. We hypothesized that these species would show general convergence in scent chemistry on account of mimicry of animal carrion and faeces.

2. Materials and methods

2.1. Study species and material

C. archeri (Phallaceae) is a widespread fungus which is thought to be endemic to southern Africa and Australasia, although it is now also naturalized in Europe and North America (Dring, 1980). Fruiting bodies consist of long, red-coloured arms that become covered with brown gleba which turns black as it dries. Fruiting bodies are visited regularly by flies. During 1 h of observation of a fruiting specimen at Pinnacle Rock in the Cobham district of the Drakensberg Mountains in February 2010, one of us (SJ) observed more than 50 visits by flies (Table 1). Headspace samples of the scent of this specimen were taken in the field using the methods described in Section 2.2.

In addition to the stinkhorn, we studied the floral scent of seven angiosperm species with foul-smelling flowers typical of the sapromyiophilous syndrome (Table 1; Fig. 1c–j). We also included *Euphorbia grandicornis* Goebel ex N.E. Br. (Euphorbiaceae) for comparison because its cyathial glands are sweet-smelling, yet its flowers are often visited by blowflies along with a wide diversity of other insects (S.D. Johnson, unpublished observations). The sapromyiophilous species included five South African stapeliads in the genera *Stapelia, Orbea* and *Huernia*, a South African iris (*Ferraria crispa*) and a South American vine, *Aristolochia cymbifera* (Table 1). Flowering plants of these species were all sampled in the botanical garden of the University of KwaZulu-Natal, apart from *Orbea verrucosa* and *A. cymbifera*, which were sampled in private gardens, and *F. crispa*, which was sampled in a population growing at Noordhoek on the Cape Peninsula. Some of the study species have been reported to be pollinated by carrion flies (Table 1). In addition, we made observations of fly visits to flowers of *S. gigantea*, *S. variegata*, *Stapelia leendertziae*, *H. hystrix*, and *F. crispa*. Identifications of the fungus and plant taxa were based primarily on the publications of de Vos (1979), Dring (1980) and Bruyns (2005). Vouchers of the plant species are deposited in the Bews Herbarium at the University of KwaZulu-Natal. Insect specimens are currently deposited in the collection at the University of KwaZulu-Natal.

2.2. Scent collection

Samples were collected using dynamic headspace extraction methods and were analysed by coupled gas chromatography and mass spectrometry (GC-MS). In total, 29 samples from nine species (Table 1) and other material (dog and horse faeces, rat carcass, rotten meat) were sampled and analysed between March 2007 and May 2010. Headspace samples were taken by enclosing fungal or plant parts in polyacetate bags (Kalle Bratschlauch Wiesbaden, Germany) prior to sampling and

Table 1
Sapromyiophilous fungus and angiosperm species investigated in this study and their insect visitors (identified to family).

Family	Species	Insect visitors (approximate number of observations)	Reference
Clathraceae	*Clathrus archeri* (Berk.) Dring	Calliphoridae (10), Muscidae (50), Sarcophagidae (20)	This study
Apocynaceae	*Stapelia gigantea* N.E.Br.	Calliphoridae (>50), Sarcophagidae (2)	(Vogel, 1954; Herrera and Nassar, 2009). This study
Apocynaceae	*Orbea variegata Haw.*	Calliphoridae (5), Sarcophagidae (3)	(Meve and Liede, 1994). This study
Apocynaceae	*Orbea verrucosa (Masson) L.C.Leach*	–	–
Apocynaceae	*Stapelia leendertziae* N.E.Br.	Calliphoridae (3), Sarcophagidae (2)	This study
Apocynaceae	*Huernia hystrix* N.E.Br.	Unidentified fly eggs and maggots in flowers	This study
Iridaceae	*Ferraria crispa* Burm.	Calliphoridae (10), Sarcophagidae (5), Scathophagidae (5)	(Goldblatt, et al., 2009), this study
Aristolochiaceae	*Aristolochia cymbifera* Mart. & Zucc.	–	–

Table 2
Average relative amounts (%) of floral scent compounds of eight sapromyiophilous plant species, the non-sapromyiophilous fly-visited species *Euphorbia grandicornis* (=*E. gra*), and other material. Scent compounds are listed according to compound class and Kovats retention index (KRI). tr = trace amount (< 0.1% of total peak area). Compound identification criteria and notes: a = comparison of MS with published data; b = comparison of MS and retention time with published data; c = comparison of MS and retention time with authentic standard. Unidentified compounds that did not reach >2% of total peak area were pooled with the number of pooled compounds in brackets. CAS = Chemical Abstracts Service Registry Number.

Compound	KRI	CAS	*C. archeri*	*A. cymbifera*	*E. grandicornis*	*F. crispa*	*H. hystrix*	*O. variegata*	*O. verrucosa*	*S. gigantea*	*S. leendertziae*	Dog faeces	Horse dung	Dead rat	Rotten meat
Number of compounds			22	63	7	23	9	10	35	14	31	18	39	14	9
Aliphatic compounds															
Aliphatic acids															
Acetic acid[b]	1479	64-19-7	3.2	–	–	1.5	–	–	–	–	–	4.0	–	–	–
Propanoic acid[b]	1556	79-09-4	1.1	tr	–	–	–	–	–	–	–	8.7	–	–	–
2-Methylpropanoic acid[b]	1602	79-31-2	1.3	–	–	–	–	–	–	–	–	4.5	–	–	–
Butanoic acid[b]	1665	107-92-6	8.6	–	–	0.4	–	–	2.4	–	–	3.3	–	–	–
3-Methylbutanoic acid[b]	1699	503-74-2	–	0.3	–	–	–	–	–	–	–	6.0	–	–	–
2-Methylbutanoic acid[a]	1689	116-53-0	8.6	–	–	–	–	–	–	–	–	–	–	–	–
Pentanoic acid[b]	1780	109-52-4	0.2	–	–	0.2	–	–	–	–	–	–	–	–	–
Hexanoic acid[c]	1863	142-62-1	–	–	–	0.6	–	–	–	–	–	–	–	–	–
Octanoic acid[b]	2087	174-07-2	–	–	–	0.5	–	–	–	–	–	–	–	–	–
Aliphatic alcohols															
Butan-1-ol[b]	1163	71-36-3	2.4	–	–	–	–	–	–	–	–	1.7	–	0.2	–
Isoamyl alcohol[b]	1221	123-51-3	0.2	–	–	–	–	–	–	–	–	0.8	–	–	–
Pentan-1-ol[b]	1258	71-41-0	–	–	–	–	–	–	–	–	–	–	0.8	tr	–
4-Methyl-1-pentanol[b]	1327	626-89-1	–	–	–	–	–	–	2.0	–	–	–	–	tr	–
(Z)-Hex-3-en-1-ol[b]	1397	928-96-1	–	–	–	–	–	–	–	0.1	–	–	2.0	–	–
Octan-3-ol[a]	1401	589-98-0	–	–	–	1.1	–	–	–	–	–	–	–	–	–
4-Methyl-1-hexanol[a]	1435	1767-46-0	–	–	–	–	–	–	4.8	–	–	–	–	–	–
Oct-1-en-3-ol[b]	1460	3391-86-4	0.4	tr	–	47.5	–	4.9	1.4	–	–	0.5	–	–	–
Heptan-1-ol[b]	1461	111-70-6	0.1	–	–	–	–	–	–	–	–	–	–	–	–
2-Ethylhexan-1-ol[a]	1502	104-76-7	–	–	–	2.4	–	–	–	–	–	–	–	–	–
(E)-Oct-2-en-1-ol[b]	1632	18409-17-1	–	–	–	1.2	–	–	–	–	–	–	–	–	–
3,7-Dimethyl-6-octen-1-ol[a]	1780	26489-01-0	–	0.1	–	–	–	–	–	–	–	–	–	–	–
Aliphatic aldehydes															
Nonanal[b]	1410	124-19-6	–	–	15.8	1.1	–	–	1.7	–	–	0.5	1.3	–	–
Decanal[b]	1516	112-31-2	–	–	20.5	0.5	3.3	–	2.7	–	–	–	1.6	–	–
Aliphatic alkanes															
Tridecane[b]	1300	629-50-5	–	–	–	–	10.0	–	–	–	–	–	–	–	–
Pentadecane[b]	1500	629-62-9	–	–	–	–	12.7	–	–	–	–	–	–	–	–
Aliphatic esters															
Isopropyl propionate[a]	1027	637-78-5	–	–	–	–	–	–	0.8	–	–	–	–	–	–
Methyl hexanoate[b]	1206	106-70-7	–	–	–	–	–	–	–	–	0.1	–	–	–	–
Isoamyl propionate[b]	1207	105-68-0	–	0.1	–	–	–	–	–	–	–	–	–	–	–
Propyl butyrate[b]	1237	105-66-8	–	tr	–	–	–	–	–	–	–	–	–	–	–
Isopropyl hexanoate[a]	1250	2311-46-8	–	–	–	–	–	–	–	–	0.3	–	–	–	–
Isopropyl tiglate[a]	1252	1733-25-1	–	–	–	–	–	–	5.0	–	–	–	–	–	–
Methyl 3-hexenoate[a]	1277	2396-78-3	–	–	–	–	–	–	–	–	0.1	–	–	–	–
Isoamyl butyrate[a]	1283	106-27-4	–	2.2	–	–	–	–	–	–	–	–	–	–	–
2-Methylbutyl 2-methylbutyrate[a]	1296	2445-78-5	–	0.1	–	–	–	–	–	–	–	–	–	–	–
Isopentyl isovalerate[a]	1306	659-70-1	–	0.5	–	–	–	–	–	–	–	–	–	–	–

(continued on next page)

Table 2 (*continued*)

Compound	KRI	CAS	*C. archeri*	*A. cymbifera*	*E. grandicornis*	*F. crispa*	*H. hystrix*	*O. variegata*	*O. verrucosa*	*S. gigantea*	*S. leendertziae*	Dog faeces	Horse dung	Dead rat	Rotten meat
Number of compounds			22	63	7	23	9	10	35	14	31	18	39	14	9
Aliphatic compounds															
Aliphatic esters															
Methyl 2-hexenoate[a]	1308	2396-77-2	–	–	–	–	–	–	–	–	1.0	–	–	–	–
Amyl 2-methylbutyrate[a]	1344	68039-26-9	–	tr	–	–	–	–	–	–	–	–	–	–	–
Isopentyl pentanoate[a]	1375	2050-09-1	–	1.1	–	–	–	–	–	–	–	–	–	–	–
Butyl (2E)-2-hexenoate[a]	1448	54411-16-4	–	–	–	–	–	–	–	–	tr	–	–	–	–
Isoamyl hexanoate[a]	1472	2198-61-0	–	0.4	–	–	–	–	–	–	–	–	–	–	–
Isopentyl heptanoate[a]	1569	109-25-1	–	tr	–	–	–	–	–	–	–	–	–	–	–
Unidentified aliphatic esters			–	0.2(3)	–	–	–	–	–	–	–	–	–	0.2(1)	–
Aliphatic ketones															
Octan-3-one[a]	1275	106-68-3	–	–	–	6.8	–	–	–	–	–	–	–	–	3.4
Acetoin[b]	1305	512-86-0	–	–	–	–	–	–	–	–	–	–	–	–	23.7
Nonan-2-one[b]	1408	821-55-6	–	0.2	–	–	–	–	–	–	–	–	–	–	4.2
4-Octen-3-one[a]	1487	14129-48-7	–	–	–	–	–	–	–	–	tr	–	–	–	–
2-Decanone[a]	1506	693-54-9	–	tr	–	–	–	–	–	–	–	–	–	–	–
Benzenoids and phenylpropanoids															
Anisole[b]	1367	100-66-3	–	–	–	–	–	–	3.7	0.6	–	–	–	–	–
Benzaldehyde[c]	1545	100-52-7	0.4	0.1	31.9	1.3	0.7	6.3	2.1	0.2	0.1	1.2	0.5	–	–
Methylbenzoate[c]	1650	93-58-3	–	tr	–	–	–	–	–	–	tr	0.6	–	–	–
Isopropyl benzoate[a]	1680	939-48-0	–	–	–	–	–	–	1.6	–	–	–	–	–	–
Benzyl acetate[c]	1751	140-11-4	–	tr	–	–	–	–	0.6	–	–	–	–	–	–
Isopropyl phenylacetate[a]	1780	4861-85-2	–	–	–	–	–	–	1.6	–	–	–	–	–	–
Benzyl isobutanoate[a]	1805	103-28-6	–	0.4	–	–	–	–	–	–	–	–	–	–	–
Benzyl propionate[a]	1817	122-63-4	–	3.8	–	–	–	–	–	–	–	–	–	–	–
Phenylethyl acetate[b]	1839	103-45-7	–	–	–	–	–	–	0.7	–	–	–	–	–	–
Methyl 3-phenylpropanoate[a]	1871	103-25-3	tr	–	–	–	–	–	–	–	–	–	–	–	–
2-Methoxyphenol[b]	1884	90-05-1	–	–	–	25.6	–	–	–	–	–	–	–	–	–
Benzyl 3-methyl butanoate[a]	1893	103-38-8	–	14.6	–	–	–	–	–	–	–	–	–	–	–
Benzyl alcohol[c]	1899	100-51-6	0.5	18.0	5.9	0.3	0.4	4.5	tr	0.1	–	0.7	0.5	tr	0.5
Benzyl butanoate[a]	1924	103-37-7	–	14.3	–	–	–	–	–	–	–	–	–	–	–
Phenylethyl alcohol[c]	1938	60-12-8	tr	0.1	–	–	0.4	8.0	2.2	tr	–	0.5	0.1	tr	1.9
2-Phenylethyl butanoate[a]	1989	103-52-6	–	0.1	–	–	–	–	0.4	–	–	–	–	–	–
Phenylethyl isovalerate[a]	1996	140-26-1	–	–	–	–	–	6.1	–	–	–	–	–	–	–
Benzyl pentanoate[a]	1999	10361-39-4	–	3.8	–	–	–	–	–	–	–	–	–	–	–
Phenol[b]	2030	108-95-2	2.3	–	5.5	0.8	–	–	7.0	0.1	0.6	58.9	0.2	tr	–
3-Phenylpropanol[a]	2071	122-97-4	–	–	–	–	–	–	–	–	–	–	tr	–	–
Benzyl hexanoate[a]	2093	6938-45-0	–	1.4	–	–	–	–	–	–	–	–	–		–

p-Cresol[b]	2100	106-44-5	4.4	2.6	–	–	–	–	1.0	0.0	–	–	5.7	tr	–
m-Cresol[b]	2107	108-39-4	–	–	–	–	–	–	–	–	–	–	17.7	–	–
4-Ethylphenol[b]	2191	123-07-9	–	–	–	–	–	–	–	–	–	–	0.2	–	–
Benzyl heptanoate[a]	2198	5454-21-7	–	0.1	–	–	–	–	–	–	–	–	–	–	–
Phenylethyl tiglate[a]	2215	55719-85-2	–	–	–	–	–	–	0.4	–	–	–	–	–	–
Benzyl benzoate[b]	2643	120-51-4	–	tr	–	–	–	–	–	–	–	–	–	–	–
Unidentified benzenoids			tr(1)	–	–	–	–	–	0.5(1)	–	–	–	–	–	–
Monoterpenoids															
α-Pinene[c]	1092	80-56-8	–	–	–	–	–	–	30.9	–	–	–	–	–	–
Sabinene[b]	1160	3387-41-5	–	–	–	–	–	–	11.5	–	–	–	–	–	–
p-Mentha-1(7),8-diene[a]	1198	499-97-8	–	–	–	–	–	–	–	–	–	–	13.6	–	–
Limonene[c]	1228	138-86-3	–	–	–	0.2	–	–	–	–	–	–	39.5	–	–
(Z)-Ocimene[b]	1257	3338-55-4	–	2.2	–	–	–	–	3.8	12.6	–	–	–	–	–
(E)-Ocimene[b]	1275	3779-61-1	–	0.3	–	–	–	–	–	67.5	–	–	–	–	–
Camphor[a]	1543	76-22-2	–	–	–	–	–	–	–	–	tr	–	–	–	–
β-Linalool[b]	1556	78-70-6	–	0.1	1.4	1.4	–	–	0.7	0.1	0.2	–	–	–	–
(E)-p-Mentha-2,8-dien-1-ol[a]	1642	7212-40-0	–	–	–	–	–	–	–	–	–	–	0.4	–	–
β-Cyclocitral[b]	1647	432-25-7	–	tr	–	–	–	–	0.3	–	–	–	0.2	–	–
Geraniol[b]	1866	106-24-1	0.1	–	–	–	–	–	–	–	–	–	–	–	–
Unidentified monoterpenoids			–	–	–	0.3(1)	–	–	–	0.1(1)	–	–	–	–	–
Sesquiterpenoids															
α-Cubebene[b]	1473	17699-14-8	–	tr	–	–	–	–	–	–	–	–	–	–	–
Bicycloelemene[a]	1499	32531-56-9	–	tr	–	–	–	–	–	–	–	–	–	–	–
α-Copaene[b]	1515	3856-25-5	–	0.1	–	–	–	–	–	–	–	–	–	–	–
γ-Gurjunene[a]	1541	22567-17-5	–	–	–	–	–	–	–	–	–	–	0.3	–	–
β-Cubebene[b]	1563	13744-15-5	–	tr	–	–	–	–	–	–	–	–	–	–	–
Aristolene[a]	1596	6831-16-9	–	0.4	–	–	–	–	–	–	–	–	–	–	–
β-Elemene[b]	1613	515-13-9	–	0.1	–	–	–	–	–	–	–	–	–	–	–
β-Gurjunene[b]	1618	17334-55-3	–	3.2	–	–	–	–	–	–	–	–	–	–	–
β-Caryophyllene[b]	1622	87-44-5	–	0.8	–	–	–	–	–	–	–	–	3.7	–	–
γ-Elemene[b]	1666	29873-99-2	–	tr	–	–	–	–	–	–	–	–	–	–	–
α-Humulene[b]	1697	6753-98-6	–	tr	–	–	–	–	–	–	–	–	1.1	–	–
α-Amorphene[b]	1715	483-75-0	–	–	–	–	–	–	–	–	–	–	0.4	–	–
Germacrene D[b]	1738	23986-74-5	–	0.2	–	–	–	–	–	–	–	–	–	–	–
Bicyclogermacrene[b]	1763	67650-90-2	–	0.6	–	–	–	–	–	–	–	–	–	–	–
α-Farnesene[b]	1771	502-61-4	–	–	–	0.9	–	–	tr	–	–	–	–	–	2.1
Cuparene[b]	1857	16982-006	–	–	–	–	–	–	0.2	–	–	–	–	–	–
Germacrene B[b]	1866	15423-57-1	–	tr	–	–	–	–	–	–	–	–	–	–	–
Cedrane[a]	2280	13567-54-9	–	–	–	–	–	–	–	–	–	–	0.3	–	–

(continued on next page)

Table 2 (*continued*)

Compound	KRI	CAS	*C. archeri*	*A. cymbifera*	*E. grandicornis*	*F. crispa*	*H. hystrix*	*O. variegata*	*O. verrucosa*	*S. gigantea*	*S. leendertziae*	Dog faeces	Horse dung	Dead rat	Rotten meat
Number of compounds			22	63	7	23	9	10	35	14	31	18	39	14	9
Aliphatic compounds															
3,7,11-Trimethyl-2,6, 10-dodecatrien-1-ol[a]	2355	4602-84-0	–	–	–	–	–	–	–	–	–	0.1	–	–	–
Unidentified sesquiterpenoids			–	0.2(9)	–	–	–	–	1.3(2)	–	tr(1)	–	5.8(12)	–	–
Diterpenes															
Verticiol[a]	2232	70000-19-0	–	–	–	–	9.4	–	–	–	–	–	–	–	–
Irregular terpenes															
6-Methyl-5-hepten-2-one[b]	1356	110-93-0	1.7	0.2	–	4.5	–	–	4.6	–	0.9	2.2	3.4	–	–
Nitrogen-containing compounds															
2,5-Dimethylpyrazine[b]	1336	123-32-0	–	–	–	–	–	11.2	–	–	0.8	–	–	–	–
2,3,5-Trimethylpyrazine[b]	1417	14667-55-1	–	–	–	–	–	–	–	–	–	–	–	–	13.7
2,3,5,6-Tetramethylpyrazine[a]	1487	1124-11-4	–	–	–	–	–	–	–	–	–	–	–	–	11.3
Benzonitrile[b]	1635	100-47-0	–	–	–	–	–	0.6	0.1	–	–	–	–	–	–
2-Phenylacetonitrile[a]	1954	140-29-4	–	–	19.0	–	–	–	–	–	–	–	–	–	–
Indole[c]	2455	120-72-9	3.0	3.4	–	–	–	28.4	3.3	0.1	tr	5.3	0.3	0.3	–
Skatole[b]	2508	83-34-1	–	–	–	–	–	–	0.7	–	–	–	0.1	–	–
Sulphur-containing compounds															
Dimethyl disulfide[c]	1124	624-92-0	21.0	19.4	–	–	32.9	13.9	–	12.3	61.6	0.5	–	52.2	39.1
Methane n-thiolbutyrate[a]	1221	2432-51-1	–	–	–	–	–	–	–	–	tr	–	–	–	–
2,4-Dithiapentane[a]	1317	1618-26-4	–	–	–	–	–	–	–	–	0.1	–	–	tr	–
Dimethyl trisulfide[c]	1407	3658-80-8	40.5	3.4	–	–	30.1	16.0	–	6.1	30.5	–	–	46.9	–
Methanethiol caproate[a]	1419	2432-77-1	–	–	–	–	–	–	–	–	0.9	–	–	–	–
2-Methylthiazolidine[a]	1454	24050-16-6	–	–	–	0.9	–	–	–	–	–	–	–	–	–
Methyl methylthiomethyl disulfide[a]	1692	42474-44-2	–	tr	–	–	–	–	–	–	tr	–	–	tr	–
S-Methyl thiobenzoate[a]	1958	5925-68-8	–	–	–	–	–	–	–	–	tr	–	–	–	–
Benzothiazole[b]	1992	95-16-9	–	–	–	0.2	–	–	–	–	–	–	–	–	–
Unidentified sulphur compounds			–	0.2(1)	–	–	–	–	0.2(1)	–	tr(1)	–	0.1(1)	tr(1)	–
Unknowns															
Other unknowns			–	–	–	–	–	–	–	–	2.6(8)	–	0.5(1)	–	–

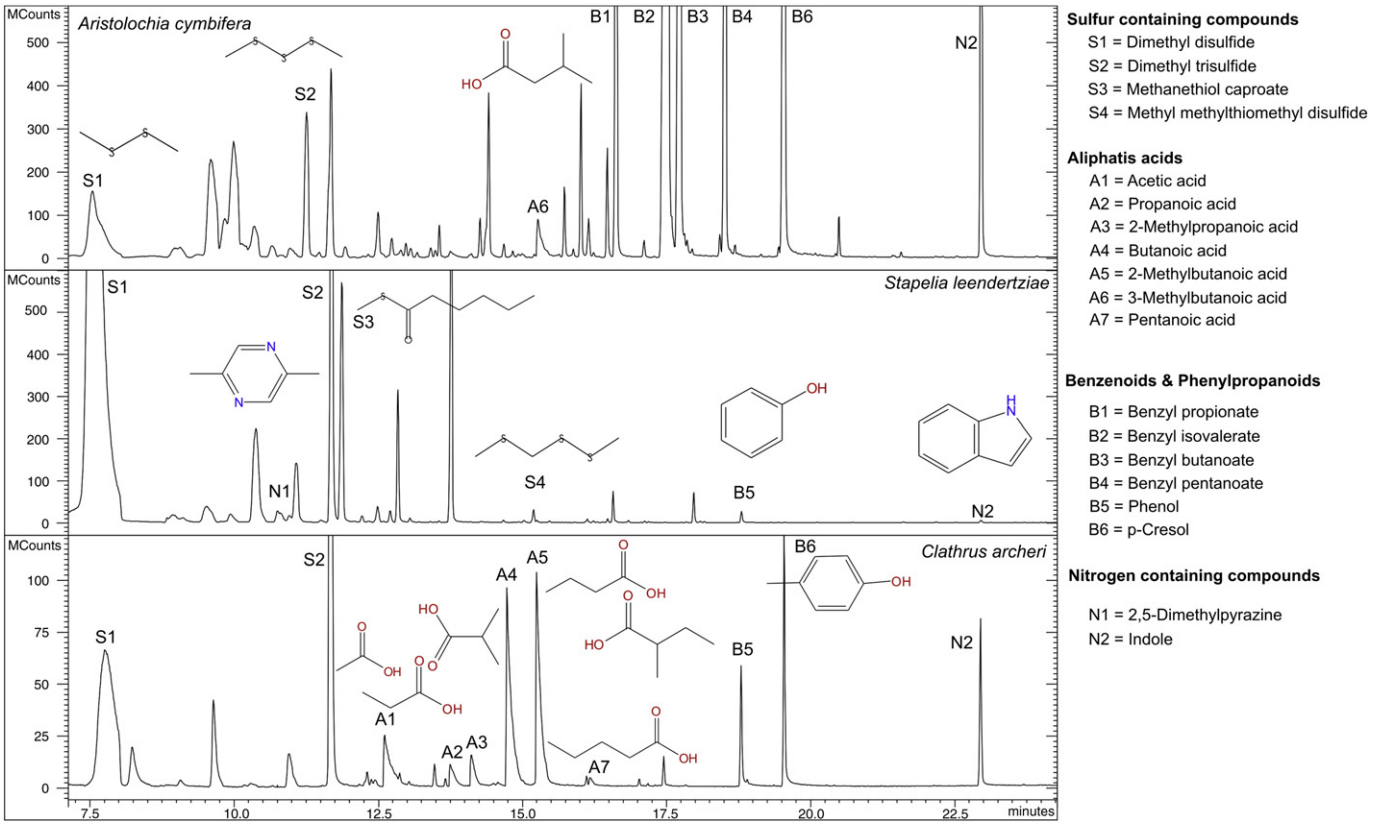

Fig. 2. Chromatograms of the volatile compounds emitted by *Aristolochia cymbifera*, *Stapelia leendertziae*, and *Clathrus archeri*. Chemical structures shown for compounds with pungent odours.

subsequently pumping air for 20–60 min through small filters filled with 1 mg of Tenax® and 1 mg of Carbotrap® using a pump with a realized flow rate of 150 ml/min. Controls were taken from an empty polyacetate bag sampled for the same duration. Reference scent samples were collected from rotting meat, a dead rat and horse and dog faeces to compare the odour composition of these samples with that of the stinkhorn fungus and the angiosperm flowers. The meat sample consisted of approximately 100 g of beef that was left to putrefy outdoors for four days before headspace collection. The rat was an adult that had been dead for approximately five days prior to sampling. Dog faeces and horse droppings were collected within 15 min of defecation.

2.3. Gas chromatography-mass spectrometry (GC-MS) analysis of floral scent

GC-MS analysis of the samples was carried out using a Varian CP-3800 GC (Varian, Palo Alto, California) with a 30 m × 0.25 mm internal diameter (film thickness 0.25 μm) Alltech EC-WAX column coupled to a Varian 1200 quadrupole mass spectrometer in electron-impact ionization mode. Cartridges were placed in a Varian 1079 injector equipped with a Chromatoprobe thermal desorbtion device (Dötterl et al., 2005; Gordin and Amirav, 2000). The flow of helium carrier gas was 1 ml/min. The injector was held at 40 °C for 2 min with a 20:1 split and then increased to 200 °C at 200 °C/min in splitless mode for thermal desorbtion. After a 3 min hold at 40 °C, the temperature of the GC oven was ramped up to 240 °C at 10 °C/min and held there for 12 min. Compounds were identified using the Varian Workstation software with the NIST05 mass spectral library (NIST/EPA/NIH Mass Spectral Library (data version: NIST 05; MS search software version 2.0 d) and verified, where possible, using retention times of authentic standards and published Kovats indices (references in the NIST 05 library)(El-Sayed, 2009). Compounds present at similar abundance in the controls were considered to be contaminants and excluded from analysis.

2.4. Analysis of floral scent data

To establish whether scent profiles of the study species are convergent, i.e. more similar to each other than expected by chance, we compared them to published profiles of the floral scents of several confamilial species which are not pollinated by carrion flies. These data were obtained from the publications of Jürgens et al. (2010) and Shuttleworth and Johnson (2010a, 2010b). We used ANOSIM (with 10,000 random permutations) in the Primer 6 program (Clarke and Gorley, 2006) to assess the variability in the scent of the samples. We used relative amounts with respect to total peak areas (proportional abundance, excluding contaminants) because the total amount of emitted volatiles varied greatly among samples. To visualize variation among samples non-metric multidimensional scaling (NMDS)

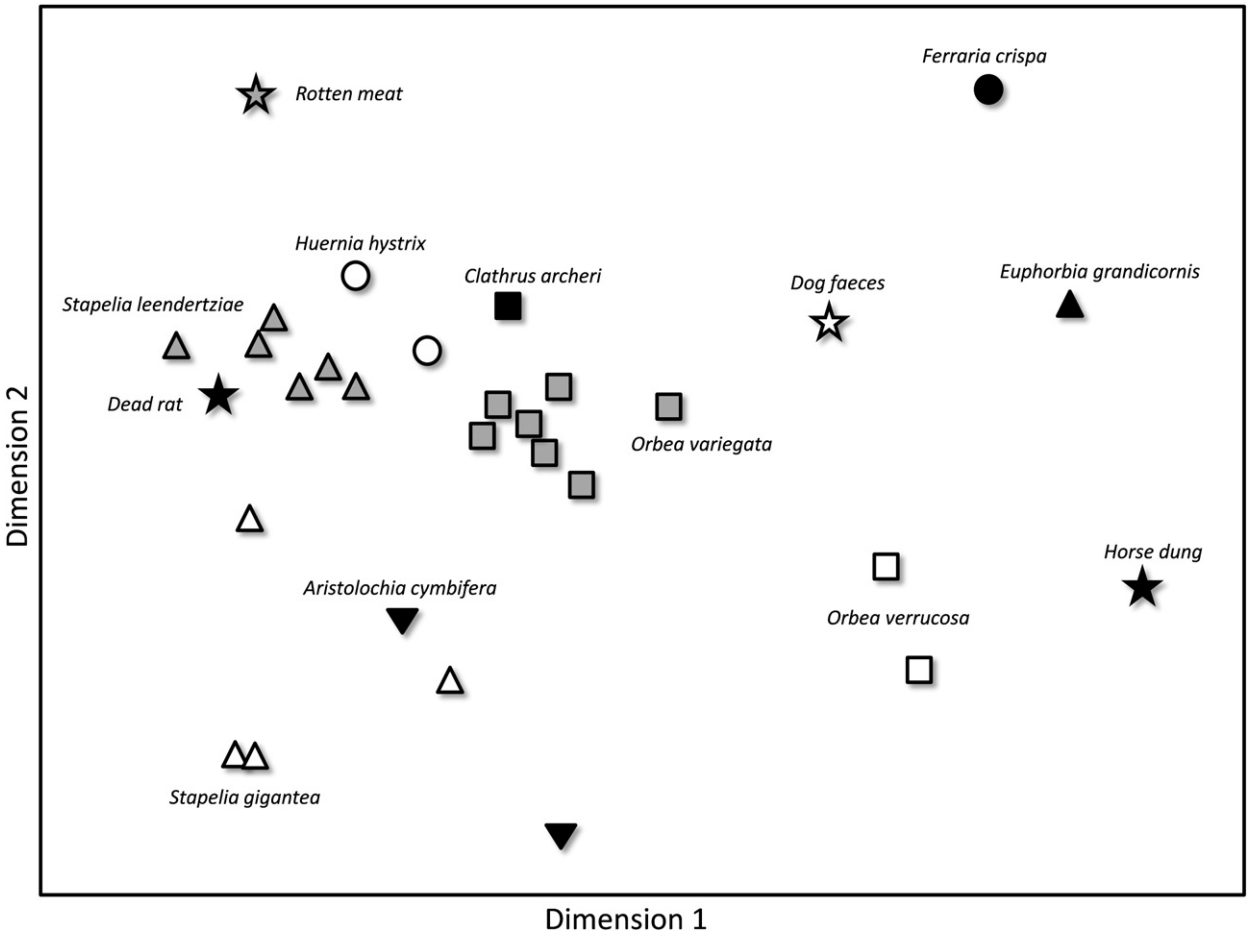

Fig. 3. Non-metric multidimensional scaling (NMDS) based on Bray–Curtis similarities of the odour composition of 29 samples representing a sapromyiophilous fungus and flowers, rotten meat, rat carcass, horse dung, and dog faeces. 2D stress value=0.15.

based on Bray–Curtis similarities was used. Data were square root transformed before calculating Bray–Curtis similarities.

3. Results

The chemical composition of the analysed scent samples is shown in Table 2. In total 152 compounds were detected, of which 123 compounds were identified. Dominant compound classes in terms of compound numbers were aliphatic compounds (49), sesquiterpenes (40), and benzenoids (29). However, sesquiterpenoids were found in low relative amounts only (all <4.0% relative amount), and mainly in the floral scent of *A. cymbifera* and horse dung. Other compound classes detected were monoterpenes (13), sulphur-containing compounds (12), nitrogen-containing compounds (7), diterpenes (1), and irregular terpenes (1). The number of compounds ranged from 63 in *A. cymbifera* to 9 in *H. hystrix* and the rotten meat sample.

The rotten meat sample and the dead rat sample were both characterized by high relative amounts of dimethyl disulphide. The main differences between these two samples were the high relative content of dimethyl trisulphide in the dead rat sample, a compound not found in the rotten meat sample, and the occurrence of acetoin and two pyrazines (2,3,5-trimethylpyrazine, 2,3,5,6-tetramethylpyrazine) in the rotten meat sample (Table 2). Sulphur compounds were found only in very minor relative amounts in the dog faeces and horse dung samples. The horse dung sample contained several monoterpenes (e.g. limonene, p-mentha-1(7),8-diene) and sesquiterpenes (β-caryophyllene, α-humulene) that were not present in any of the other samples with biological material. The dog faeces sample was dominated by phenol (58.9%), several aliphatic acids (e.g. acetic acid, propanoic acid, butanoic acid), and the nitrogen-containing compound indole (5.3%).

Sulphur-containing compounds were, in terms of relative amounts, the most important compound class. The scent of *S. leendertziae* consisted of more than 90% dimethyl disulphide (DMDS) and dimethyl trisulphide (DMTS). Other species with high relative amounts of DMDS and DMTS were the stinkhorn fungus *C. archeri* (61.5%), and the two stapeliads, *H. hystrix* (63.0%) and *Orbea variegata* (29.9%). A high relative amount of DMDS was also found in the rotten meat sample (39.1%).

A comparison of the chromatogram of the stinkhorn fungus *C. archeri* with that of *A. cymbifera* and *S. leendertziae* (Fig. 2) shows that the three species have distinct odour patterns. The floral scent of *A. cymbifera* contained high relative amounts of aromatic esters (e.g. benzyl propionate, benzyl butanoate, benzyl pentanoate), sulphur compounds (DMDS and DMTS),

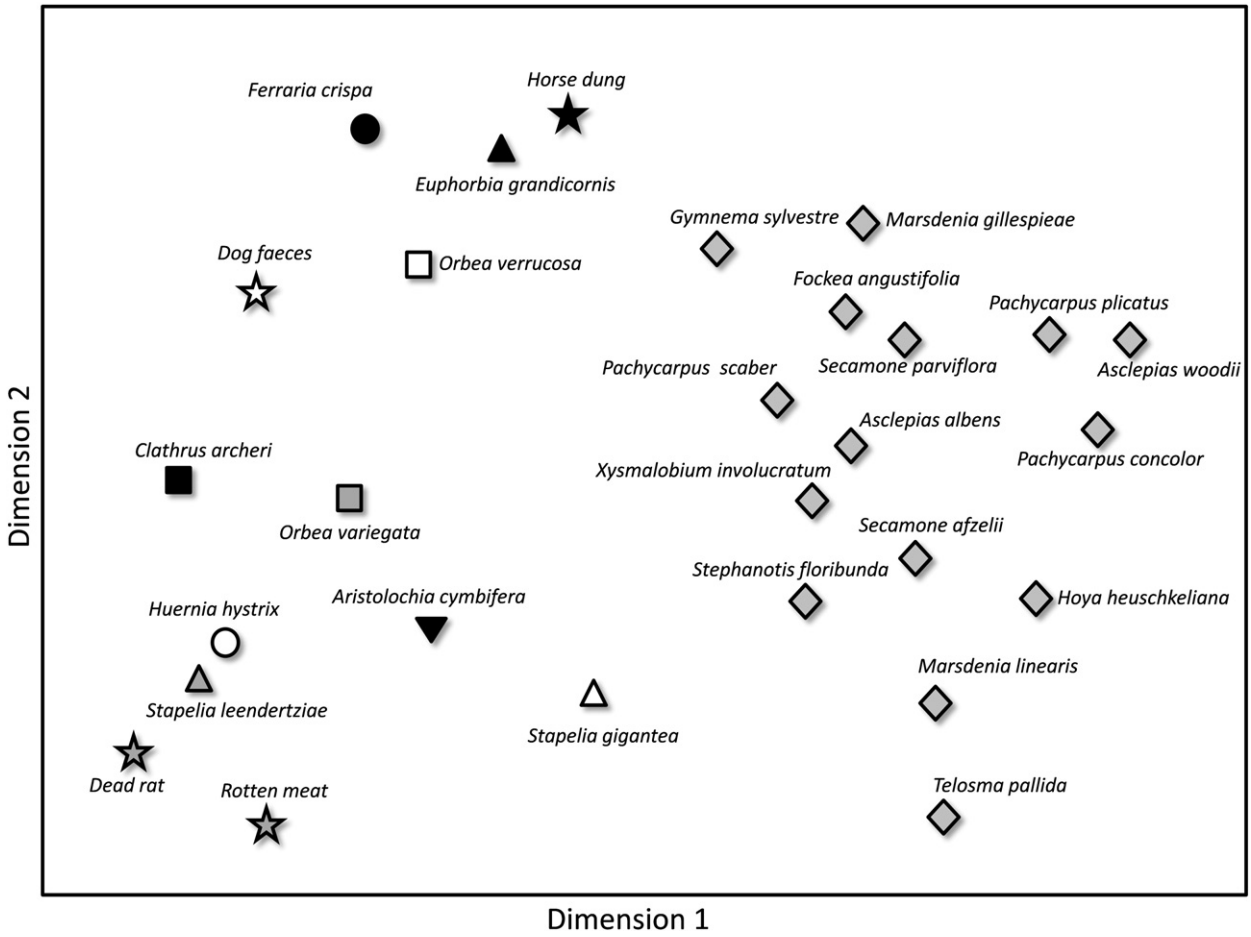

Fig. 4. Non-metric multidimensional scaling (NMDS) based on Bray–Curtis similarities of the mean odour composition of the fly-visited study species and carrion and faecal samples (symbols correspond to Fig. 2), as well as 15 confamilial species which do not have putrid odours (indicated by diamonds with grey fill). 2D stress value = 0.18.

p-cresol and indole. *S. leendertziae* flowers emitted mainly DMDS and DMTS accompanied by other sulphur-containing compounds, phenol, indole, and several aliphatic esters. The scent sample of the stinkhorn fungus *C. archeri* contained a combination of sulphur-containing compounds, phenol, p-cresol and several aliphatic acids (Table 2). This shows that the three species, despite their differences in odour composition, have a considerable amount in common, particularly in the emission of sulphur-containing compounds, indole, p-cresol, and phenol.

A two-dimensional representation of an NMDS analysis of the individual samples revealed groupings according to species (Fig. 3). There were significant overall differences in scent composition among the species included in these samples (ANOSIM, $R = 0.77$, $p < 0.01$). The odour profiles of the two stapeliad flowers *S. leendertziae* and *H. hystrix* were most similar overall to that of the rotten meat and dead rat samples. Samples of *O. variegata* and the stinkhorn fungus *C. archeri* fell in positions intermediate between the carrion and the faecal samples. In the case of the stinkhorn this is due to the occurrence of aliphatic acids (e.g. acetic acid, butanoic acid, propanoic acid; see also Fig. 2) also found in dog faeces. In *O. variegata* phenol and indole contributed mostly to the intermediate position of this species. All four species and the carrion samples were characterized by high relative amounts of DMDS and DMTS. *E. grandicornis* and *O. verrucosa* showed an intermediate position between the dog faeces sample and the horse dung sample. The odour patterns of *A. cymbifera* and *S. gigantea* occupy relatively isolated positions in the NMDS. In the case of *A. cymbifera* this is due to the occurrence of aromatic esters (Fig. 2; Table 2), while in the case of *S. gigantea* it is because two monoterpenes, (Z)-ocimene and (E)-ocimene, dominated the scent.

A striking pattern of convergence among the species with putrid odours was evident in a two-dimensional representation of an NMDS analysis that included confamilial species that lack putrid odours (Fig. 4). There were significant overall differences in odour composition between fly-visited study species, carrion and faecal samples and the non-putrid confamilial species ($R = 0.702$, $P < 0.001$). Overall odour composition in the fly-visited study species did not differ significantly from that in the carrion and faecal samples ($R = 0.04$, $P = 0.38$), while it differed significantly from the confamilial species with non-putrid odours ($R = 0.783$, $P < 0.001$).

4. Discussion

Our results support the hypothesis of convergent evolution of scents between a stinkhorn fungus and certain angiosperm flowers. The stinkhorn appears to have a scent which mimics

both carrion odours (e.g. oligosulphides) and faecal odours (e.g. phenol and p-cresol). In the NMDS analysis, the scent of this fungus was intermediate between that of rotten meat and dog faeces (Fig. 3). This is reflected in its fly visitors, which were observed to be calliphorids (which lay eggs on carrion and frequently feed on faeces), sarcophagids (which are associated with carrion), and muscids (which breed and feed on decaying organic matter, including faeces). Since spore dispersal does not require any morphological matching between the fruiting body and flies (any fly, regardless of size, that feeds on the gleba can disperse spores), there would probably be little advantage to emitting a more specific signal. The role of scent in the attraction of flies was not tested experimentally, but we found that polyacetate bags that had been used to sample scent of the fungus were strongly attractive to flies after the fungus had been removed, indicating that scent alone was sufficient for attraction.

The overall similarity in the scent profiles of the fungus and angiosperm flowers to those of rotting meat and animal faeces (Figs. 3 and 4), together with their shared fly fauna, makes it almost certain that the fungus and most of the angiosperm study species are mimics of fly brood sites and food sources. Although, the fungus produces an edible gleba and most of the studied angiosperm species produce nectar (Bruyns, 2005; Goldblatt et al., 2009), the flies are almost certainly being lured to the putrid-smelling species through exploitation of their innate attraction to decaying animal matter, rather than associative conditioning on a signal by the actual reward. The function of the gleba and nectar seems merely to ensure that spores are consumed and to orientate the flies to sexual parts of the flower in the cases of the fungi and stapeliads, respectively.

Fly visitation to the flowers of several stapeliad flowers is well-documented in a diffuse literature, much of it contributed by amateur succulent growers that observed plants in cultivation outside of their native ranges. *S. gigantea* has even become invasive in Venezuela, where it is pollinated by various carrion flies that apparently perform the same functional role in pollination as the flies in its native habitat (Herrera and Nassar, 2009). Our observations confirm that flies visit *S. gigantea* and *O. variegata* in their native ranges, in eastern and south-western South Africa, respectively. Flowers of *A. cymbifera* are visited by carrion flies in gardens where it is cultivated in South Africa. This, together with the foul smell of the flowers, which has a chemical composition similar to that of the stinkhorn and some of the stapeliads (Fig. 2), makes it highly likely that this plant is also pollinated by carrion flies in its native range.

The scent of the iris *F. crispa* was unlike most of the other species included in this study. It is dominated by 1-octen-3-ol (mushroom alcohol), a compound usually associated with fungi (e.g. Venkateshwarlu et al., 1999), various aliphatic acids and the benzenoid guiacol. Our own observations and those of Goldblatt et al. (2009) indicate that this species is visited by a typical suite of insects associated with decaying organic matter. The scent does contain phenol, a compound characteristic of faeces, but only in trace amounts. In the case of the sweet-smelling flowers of *E. grandicornis*, the basis for attraction of flies is more likely to be simple nectar-seeking and may thus involve associative conditioning. The scents of both *F. crispa* and *E. grandicornis* are outliers in the NMDS analysis (Figs. 3 and 4).

Carrion fungi and flowers appear to vary in their ability to dupe pollinators into undergoing behaviour that would normally occur on the model. In the same way as the response of male bees to sexually deceptive flowers varies from momentary attraction to lingering copulatory routines, the responses of carrion flies seem to vary among the sapromyiophilous species in this study. On *C. archeri* and *F. crispa* (Fig. 1b and i) blowflies and flesh flies appeared content to feed on the gleba and nectar, respectively, while female flies regularly laid eggs on flowers of *H. hystrix* and *S. gigantea* (Fig. 1d). It is not yet clear whether this effective deception of flies by these stapeliads is due solely to a particular scent blend or whether the hairy rugose surface of the corolla lobes plays an important additional role in eliciting the egg-laying behaviour.

This preliminary study extends our knowledge of the chemical composition of the scents of sapromyiophilous flowers and fungi. It shows that there are clear patterns of convergence across genera and even kingdoms in the emission of known fly attractants such as oligosulphides and p-cresol (Figs. 2 and 4). However, we still lack an understanding of the significance of variation in scent among closely related species, such as those in the genus *Stapelia*, and how this may relate to attraction of different groups of flies seeking either brood sites or food sources. To solve these remaining problems, priorities for future investigations will have to be the collection of more pollinator data and the use of experimentation to determine the functional significance of particular compounds and blends, as well as morphology and colour, in both the attraction of flies and the elicitation of egg-laying behaviour.

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